Deciphering the Role of the *sinR* Gene Homolog in *Halobacillus* BBL2006

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ABSTRACT

The *sinR* gene and its protein are responsible for the regulation of biofilm formation in *Bacillus subtilis*. Bacterial biofilms are important ecologically and medically, so the study of biofilm formation in the environmental isolate *Halobacillus* BBL2006 could contribute significantly to the knowledge of these bacterial systems. We have recently sequenced and annotated the whole genome of *Halobacillus* BBL2006, and identified and isolated a homolog to the *sinR* gene. We plan to mutate (knock out) the *sinR* gene in the *Halobacillus* bacteria to determine the effect upon biofilm formation in this organism.

INTRODUCTION

The moderate halophiles are a fascinating group of salt tolerant bacteria that can respond to increasing or decreasing salt concentrations in the environment. The gram positive, spore forming bacteria of the genus *Halobacillus* are representative of the moderate halophiles. Due to their tolerance of varying salt concentrations, moderate halophiles are promising candidates for the bioremediation of salt contaminated water. In addition, these bacteria can form biofilms, complex communities of bacterial cells, which may enhance their ability to bind metal contaminants. As part of our research we have characterized and sequenced the whole genome of a single *Halobacillus* (NCBI Accession: PRJNA260509 ID: 260509). Performing a BLAST search of our genome sequence has allowed us to identify a gene in our *Halobacillus* isolate which is a homolog to the *sinR* gene in *Bacillus subtilis*. The *sinR* gene of *Bacillus* encodes a protein which regulates biofilm formation in this organism. The identification and subsequent inactivation of the *sinR* gene homologue in our *Halobacillus* isolate may allow us to generate a strain which is both salt tolerant and grows as a biofilm for use in our bioremediation studies of heavy metal contaminants in water.

METHODS

- Locating the *sinR* gene homolog of *Halobacillus* BBL2006

  We used the protein Basic Local Alignment Search Tool (BLASTp) from the National Center for Biotechnology Information (NCBI) to identify the SinR protein in *Halobacillus halophilus* which is homologous to that of the SinR protein of *Bacillus subtilis*. Next, we used the nucleotide BLAST (BLASTn) to locate the *sinR* gene homolog from our whole genome sequence of *Halobacillus* BBL2006 using the nucleotide sequence of *H. halophilus*.

- Cloning Strategy
  - Clone *sinR* PCR product into pGEM T-easy vector which is a T vector designed to clone PCR product amplified with Taq polymerase
  - The pGEM + *sinR* DNA is ligated and transformed into competent *E. coli* cells
  - The cloned plasmid is extracted and digested with BglII restriction enzyme which will allow for the cutting open of the *sinR* gene where the chloramphenicol acetyltransferase (CAT) gene will be inserted using matching BglII ends
  - Use of our original primers designed for *sinR* PCR will amplify a large quantity of this *sinR*/*cat* gene construct
  - This amplified DNA will then be used to transform *Halobacillus* BBL 2006, which should result in the strain having a defective *sinR* gene due to insertional inactivation
  - Transformed cells will grow on LB agar plates supplemented with chloramphenicol antibiotic. The result that we expect is long-chain biofilm-like growth in *Halobacillus* BBL2006
- Primers were ordered from Integrated DNA Technologies
- PCR was performed using New England Biolabs (NEB) OneTaq Quick-Load 2x Master mix with standard buffer according to manufacturer protocol with an annealing temperature of 47 °C
- Restriction enzyme digests and DNA ligations were performed with NEB restriction enzymes or T4 DNA ligase according to manufacturer protocols

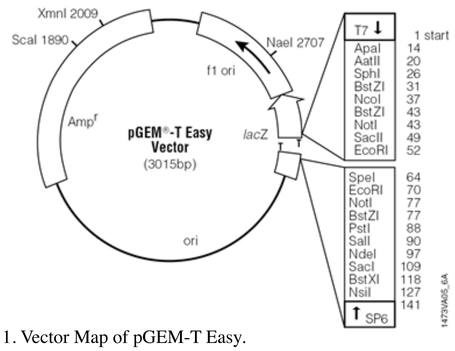
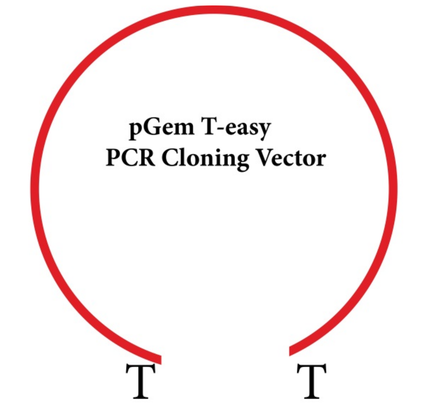


Figure 1. Vector Map of pGEM-T Easy.

CLONING STRATEGY



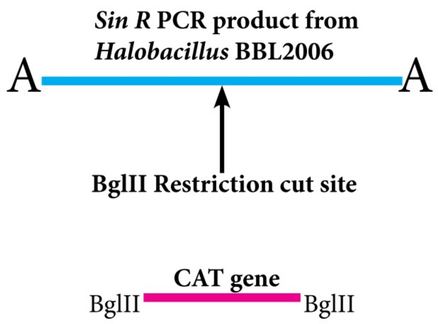


Figure 2. Cloning Strategy Model. Utilizing the BglII enzyme, the CAT gene will be inserted into the *sinR* gene PCR product ligated into the pGEM T-Easy vector

RESULTS

The *sinR* gene homolog sequence was successfully located in *Halobacillus* BBL 2006 utilizing the BLAST (NCBI). We have recovered two products of the *sinR* homolog with extra DNA upstream and downstream from the gene. The extra DNA on either side of the gene allows for additional homology which will increase the uptake of the cloned DNA containing the inactivated *sinR* gene into the *Halobacillus* BBL2006 bacteria. The uptake and incorporation of the *sinR* DNA into the chromosome of *Halobacillus* will allow for the insertional inactivation of the *sinR* gene and the ability to grow on media containing the antibiotic chloramphenicol.

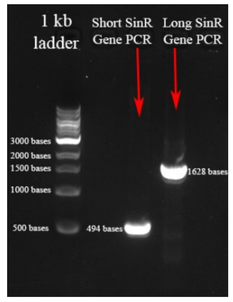


Figure 3. *sinR* Gene PCR Products. Two separate sets of PCR primers were designed to amplify the *sinR* gene with its adjacent DNA. One set allowed the addition of 154 bases on either side of the gene and the other set allowed for the addition of 1280 bases on either side of the gene.

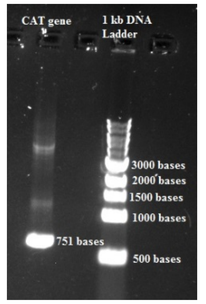


Figure 4. CAT Gene PCR Product. The chloramphenicol acetyl transferase (CAT) gene was amplified using PCR primers which were designed to allow the synthesis of BglII restriction enzyme sites on the ends of the CAT gene for ligation into the *sinR* gene.

DISCUSSION

The formation of a *Halobacillus* BBL 2006 mutant which would grow as a filamentous form, necessary to assemble into a biofilm community, may be of significant value in bioremediation of wastewater containing high salt concentrations. Currently our research is close to completion of a plasmid construct which would accomplish the inactivation of the gene of *Halobacillus* BBL2006 which is homologous to the master regulator of biofilm formation in *Bacillus subtilis*. The *sinR* gene encodes for the transcriptional regulator protein SinR which controls formation of biofilms in *B. subtilis*.
The *Halobacillus sinR* mutant will be screened for filamentous growth and used in experiments to determine its ability to remove copper contamination from wastewater at various salt concentrations.

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